

Genome-Enabled Molecular Tools for Reductive Dehalogenation

- A Shift in Paradigm for Bioremediation -

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14. ABSTRACT Chloroethenes, including vinyl chloride, are widespread groundwater pollutants. A comparative genomic analysis revealed that the vinyl chloride reductase operon, vcrABC, of Dehalococcoides sp. strain VS is embedded in a horizontally-acquired genomic island that integrated at the single-copy gene ssrA. The high similarity between vcrABC sequences from diverse Dehalococcoides is quantitatively consistent with recent horizontal acquisition driven by ~100 years of industrial pollution with chlorinated ethenes. Moreover, most rdh genes relevant for bioremediation appear to be predominantly acquired by Dehalococcoides via this mechanism. This talk will discuss the implications of these finding for development and use of molecular tools for monitoring and predicting in situ remediation of chloroethenes via anaerobic reductive dehalogenases.					
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GENOME-ENABLED MOLECULAR TOOLS FOR REDUCTIVE DEHALOGENATION

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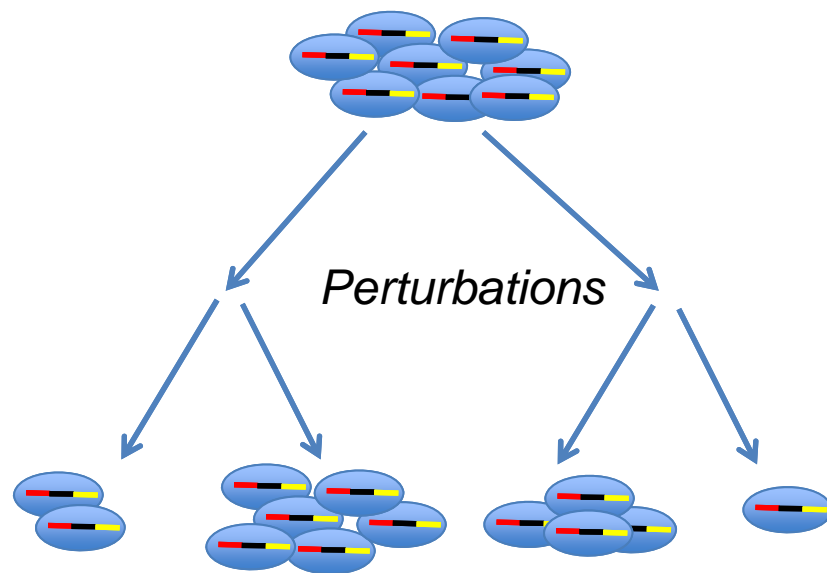
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Chloroethenes, including vinyl chloride, are widespread groundwater pollutants. A comparative genomic analysis revealed that the vinyl chloride reductase operon, *vcrABC*, of *Dehalococcoides* sp. strain VS is embedded in a horizontally-acquired genomic island that integrated at the single-copy gene *ssrA*. The high similarity between *vcrABC* sequences from diverse *Dehalococcoides* is quantitatively consistent with recent horizontal acquisition driven by ~100 years of industrial pollution with chlorinated ethenes. Moreover, most *rdh* genes relevant for bioremediation appear to be predominantly acquired by *Dehalococcoides* via this mechanism. This talk will discuss the implications of these findings for development and use of molecular tools for monitoring and predicting in situ remediation of chloroethenes via anaerobic reductive dehalogenases.

The 'old' paradigm

Bioremediation is mediated by a single
 important gene in a single strain that is
robust to environmental perturbations



 = *Dehalococcoides* sp.

 = reductive dehalogenase genes

Reductive dehalogenation of chloroethenes as a microbial community process



Reductive dehalogenation of chloroethenes as a microbial community process

Dehalobacter sp.
Desulfitobacterium sp.
Dehalospirillum sp.
Geobacter sp.
Dehalococcoides sp.

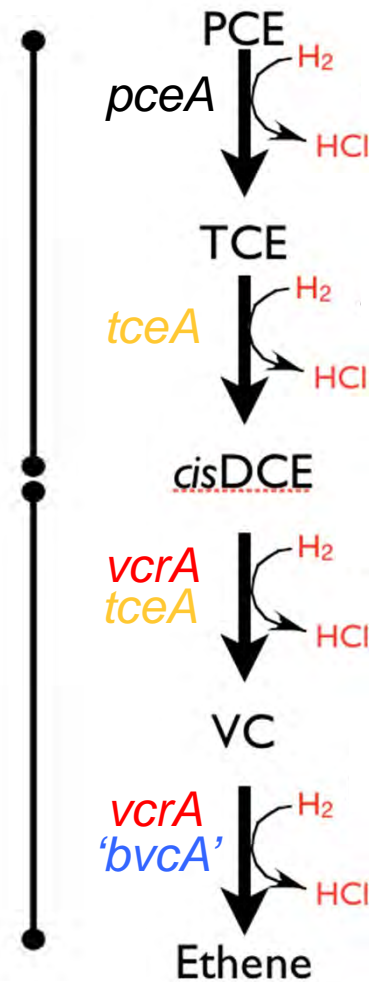
Dehalococcoides sp.

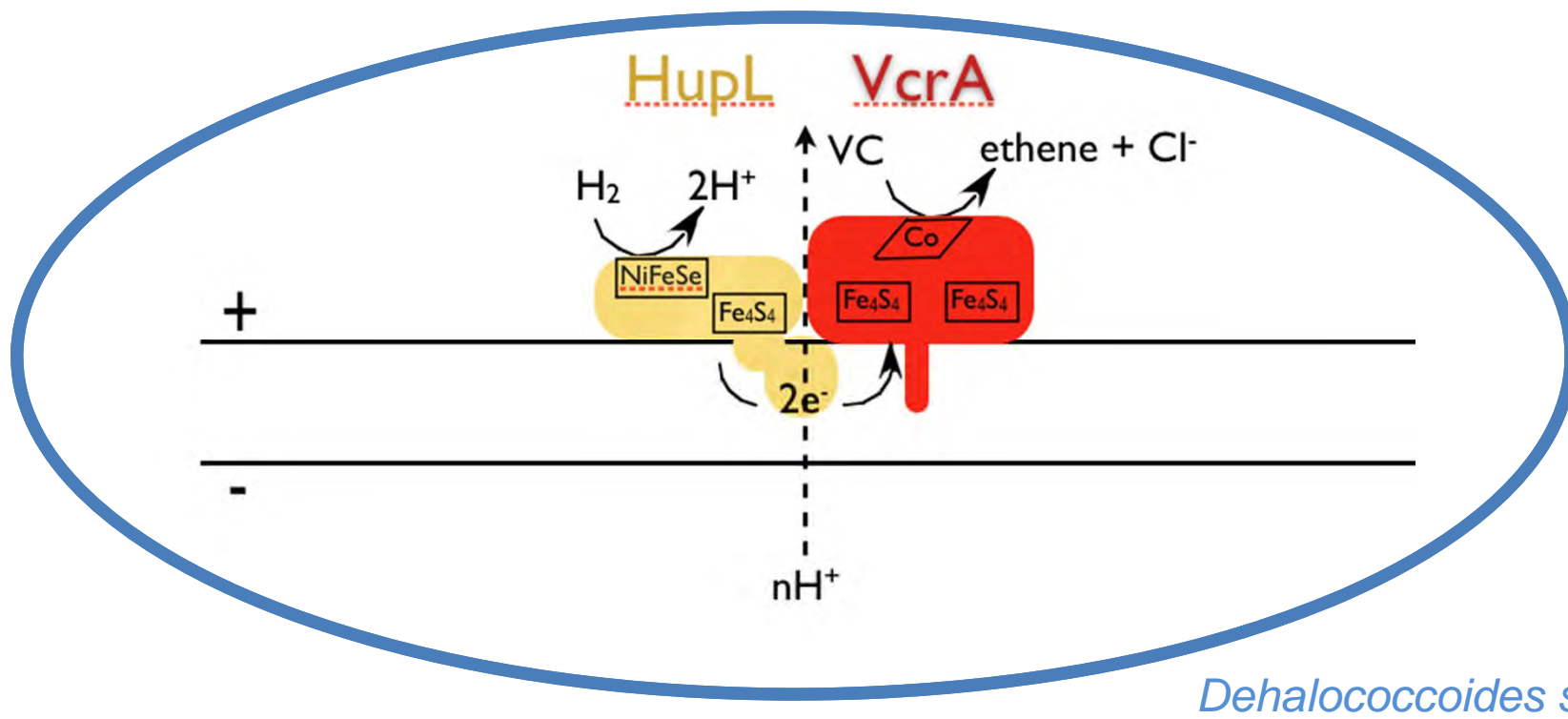


Reductive dehalogenation of chloroethenes as a microbial community process

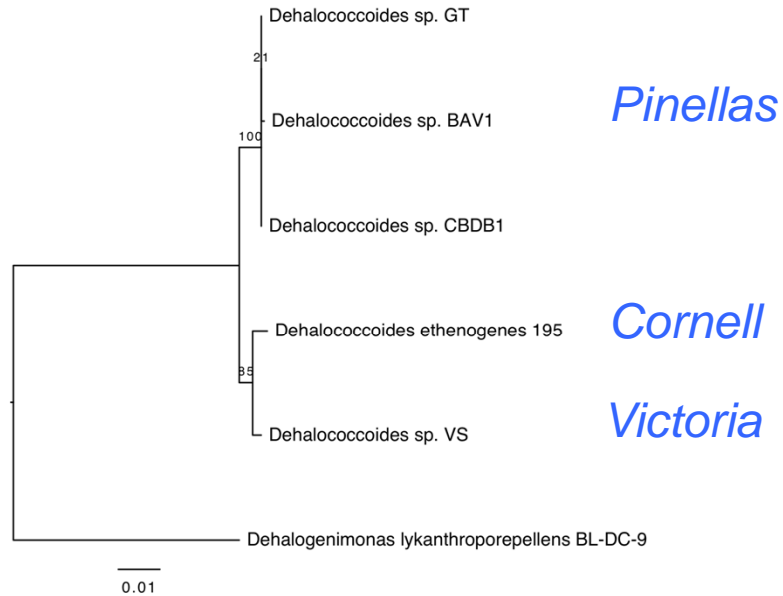
Dehalobacter sp.
Desulfitobacterium sp.
Dehalospirillum sp.
Geobacter sp.
Dehalococcoides sp.

Dehalococcoides sp.

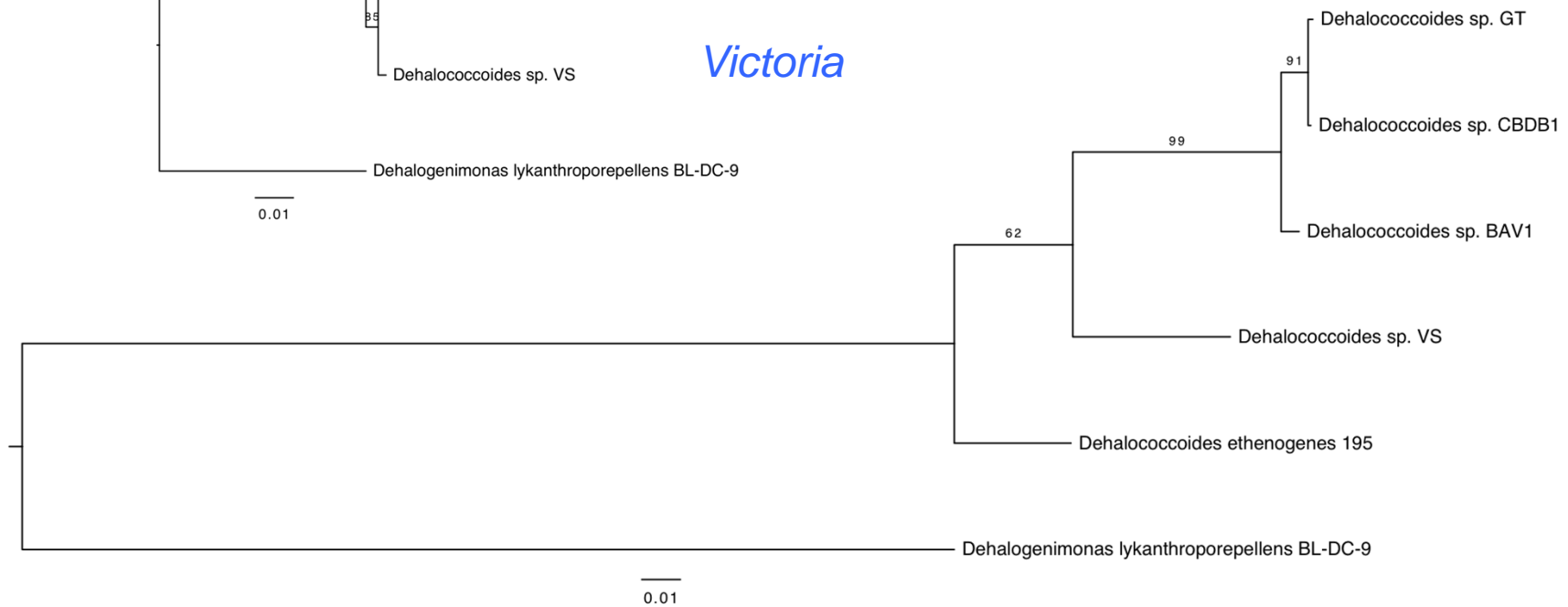




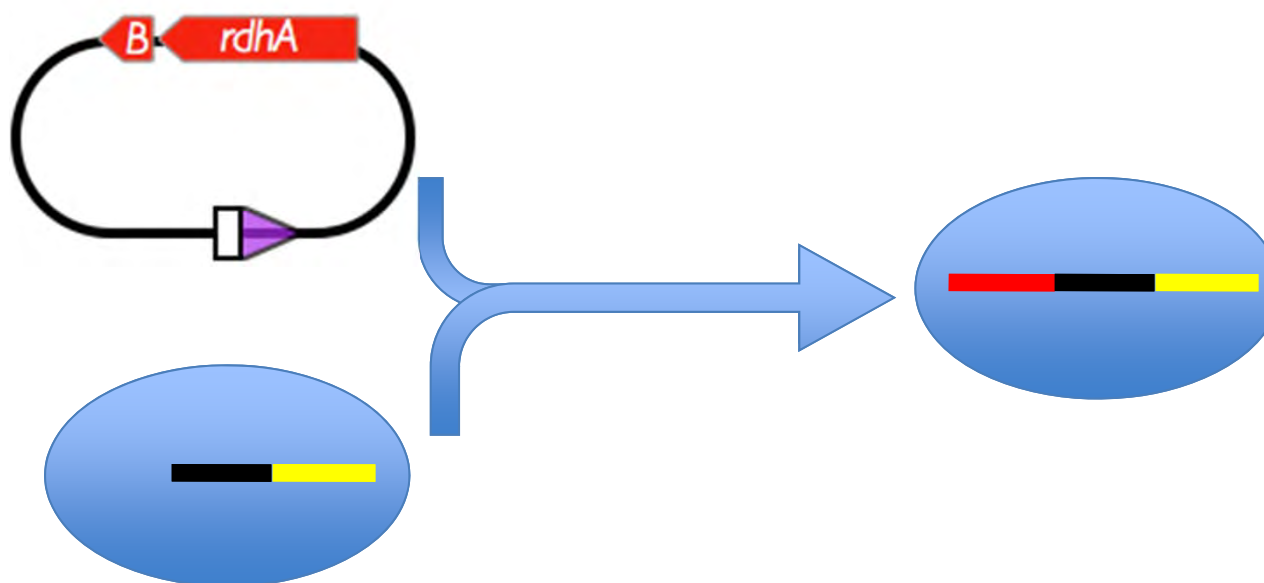
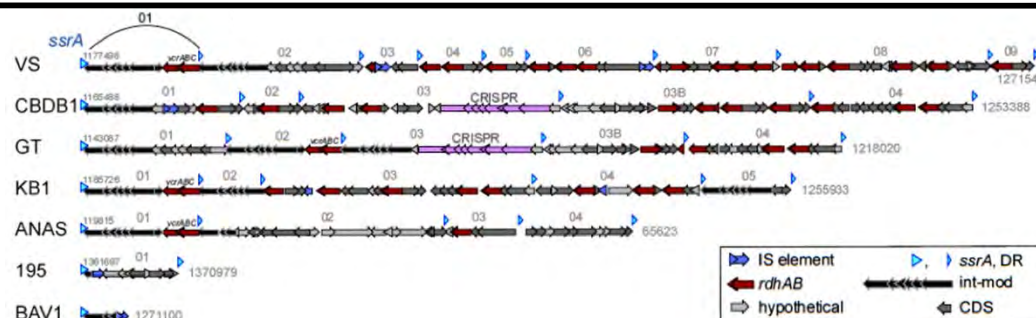
16S rRNA gene-based phylogeny



hupL-based phylogeny

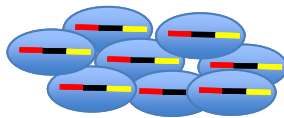


Evolution of reductive dehalogenation traits in *Dehalococcoides* sp.

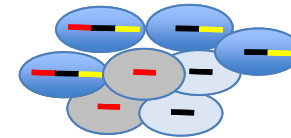


Reductive dehalogenase genes move as mobile elements in *Chloroflexi* populations

The 'old' paradigm



The new paradigm



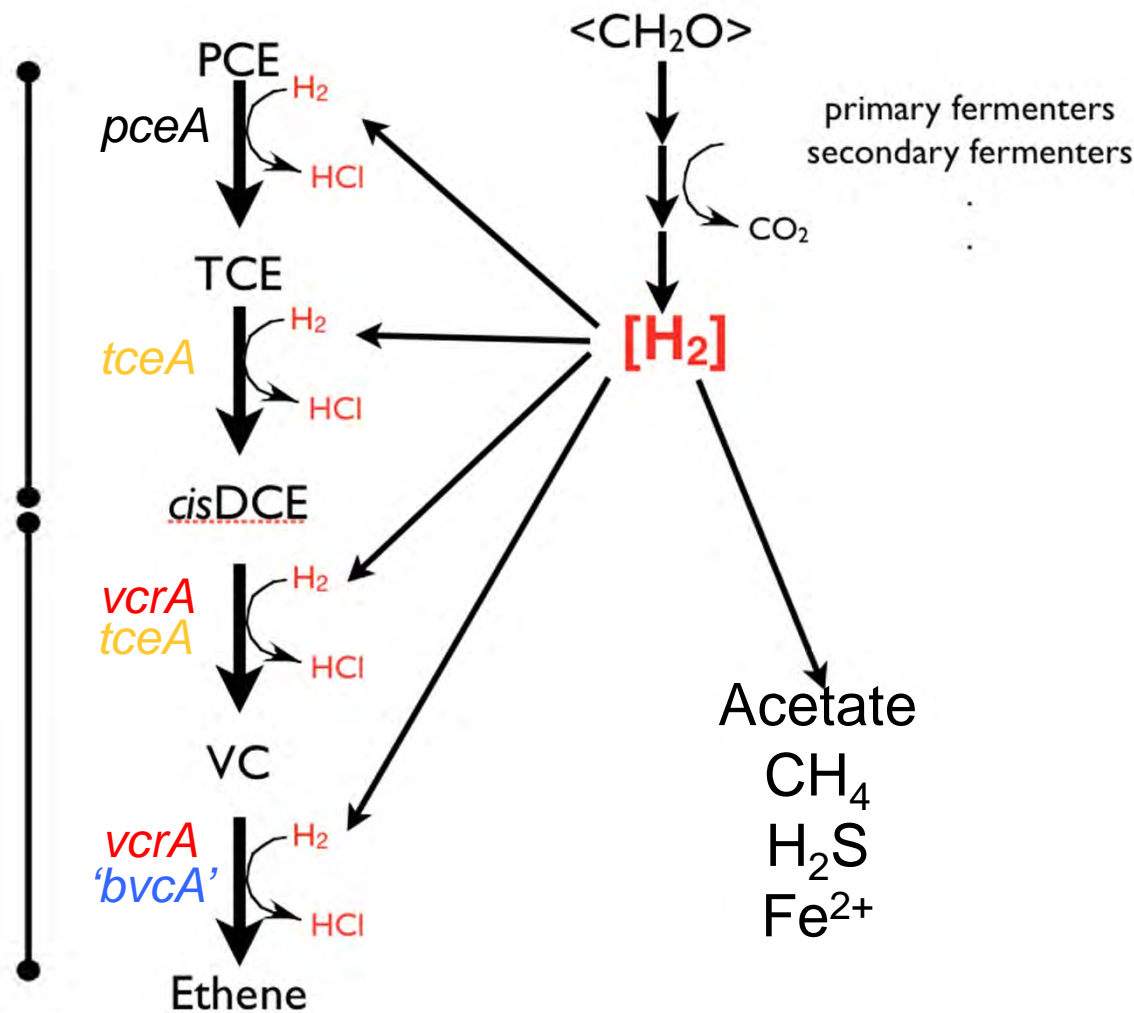
 = *Dehalococcoides* sp.

 = reductive dehalogenase genes

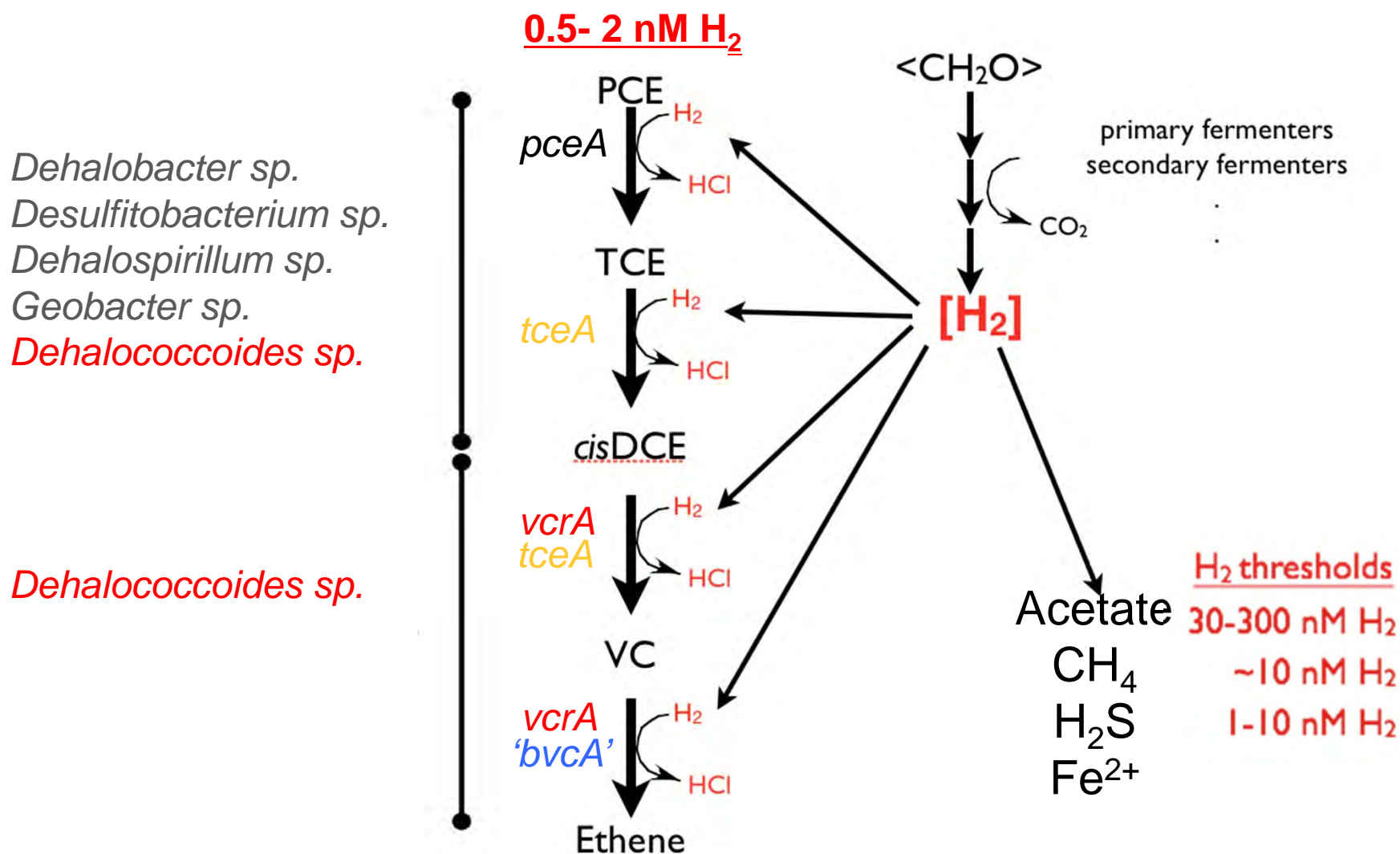
Reductive dehalogenation of chloroethenes as a microbial community process

Dehalobacter sp.
Desulfitobacterium sp.
Dehalospirillum sp.
Geobacter sp.
Dehalococcoides sp.

Dehalococcoides sp.



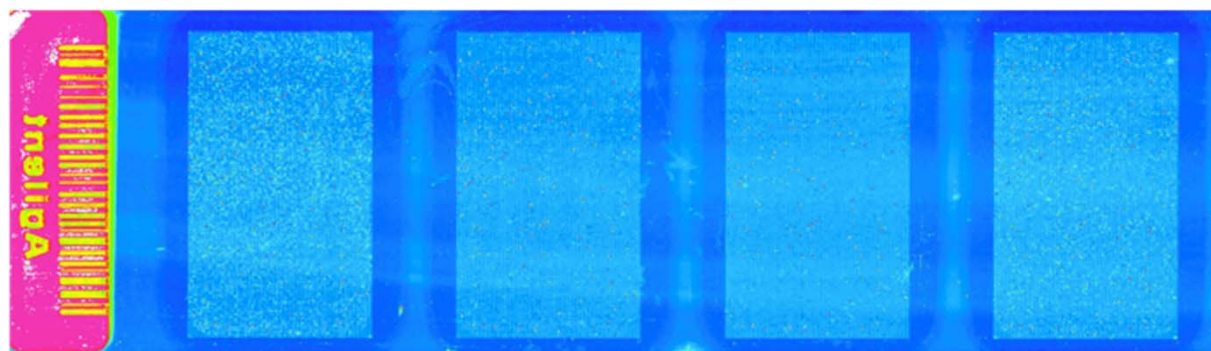
Reductive dehalogenation of chloroethenes as a microbial community process



How to assess gene dynamics and H₂ flux in heterogeneous environments?

How to assess gene dynamics and H₂ flux in heterogeneous environments?

Design of a tiled functional gene array



Hydrogenase gene array
(H₂ase Chip)

~2300 diverse
hydrogenase genes

Reductive dehalogenase gene array
(Rdh Chip)

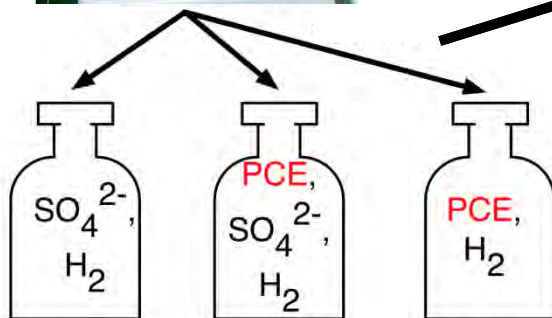
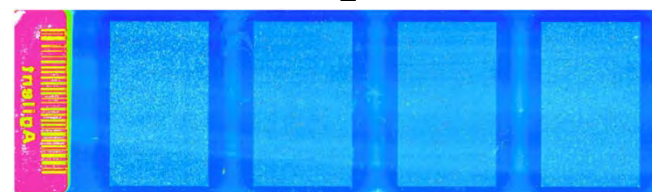
~280 diverse
rdh genes

H₂ flux in complex environments

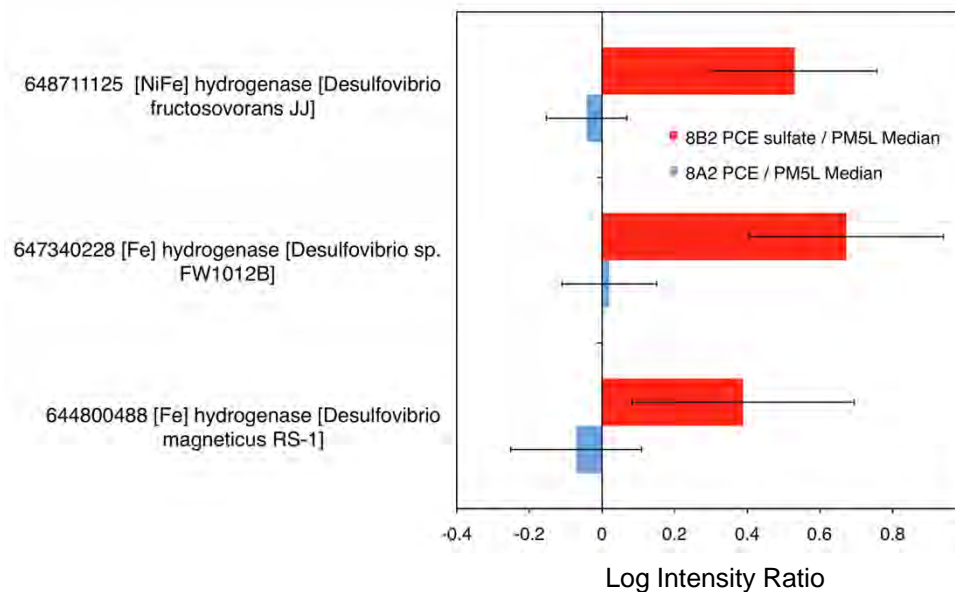
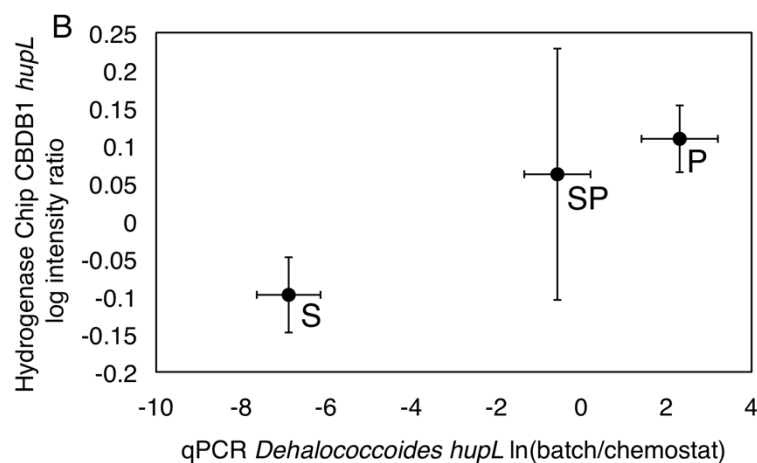
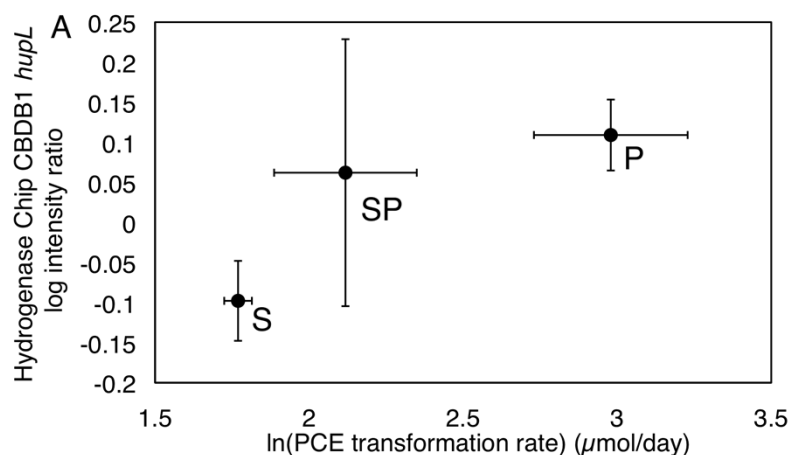
5L Lactate/PCE
Chemostat



Analysis by H₂ase gene chip

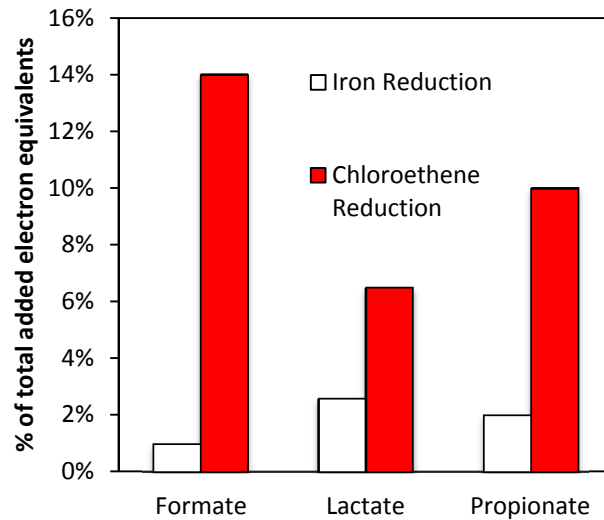
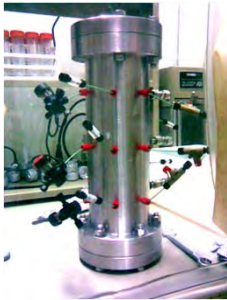


H₂ flux in complex environments

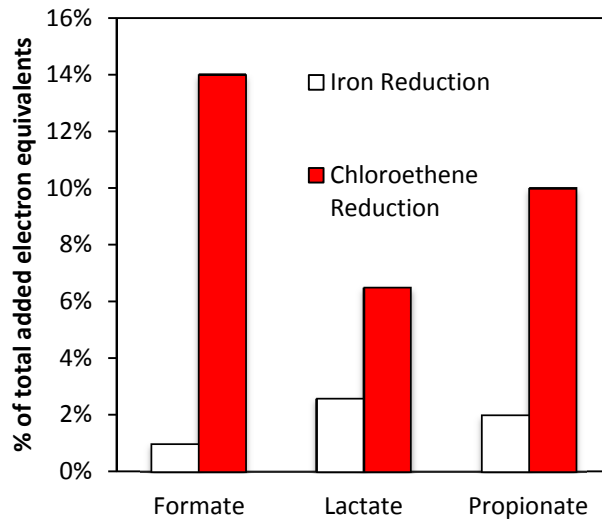


Hydrogenase chip can predict H₂ flux in complex communities

How do different (indirect) H₂ donor effect Dhc populations?



How do different (indirect) H₂ donor effect Dhc populations?



Geobacter metallireducens GS-15
[NiFe] hydrogenase

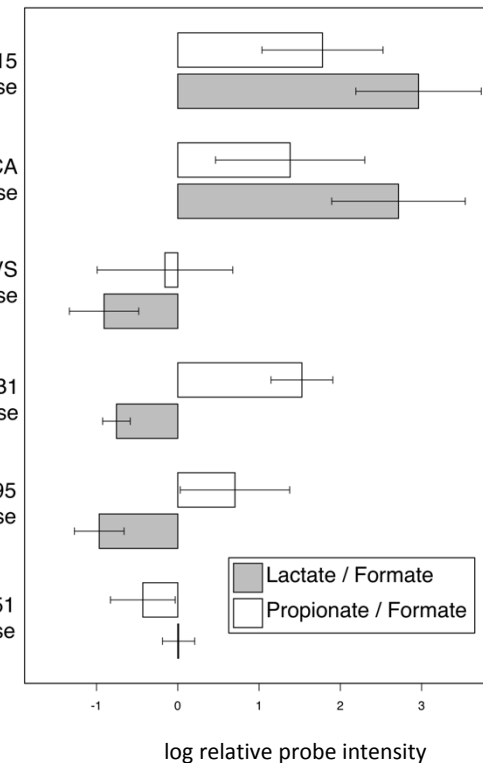
Geobacter sulfurreducens PCA
[NiFe] hydrogenase

Dehalococcoides sp. VS
hupL [NiFe] hydrogenase

Dehalococcoides sp. CBDB1
hupL [NiFe] hydrogenase

Dehalococcoides ethenogenes 195
hupL [NiFe] hydrogenase

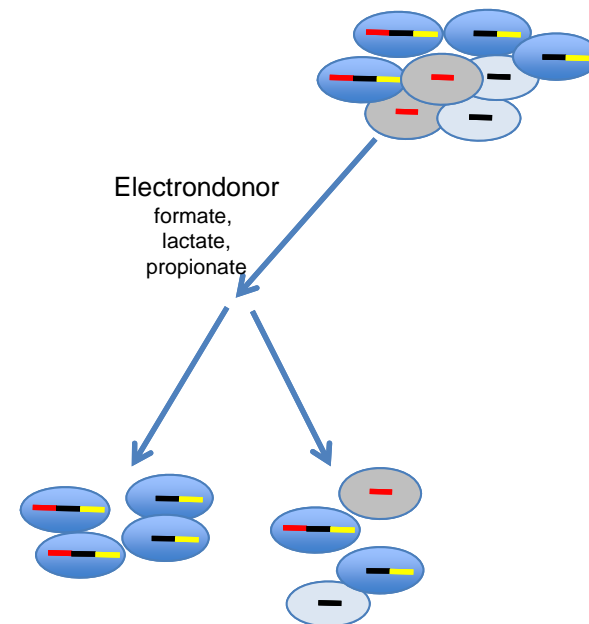
Desulfitobacterium hafniense Y51
[NiFe] hydrogenase



Dynamic changes in *Dehalococcoides* populations upon change in H₂ source

The new paradigm

Bioremediation is largely due to activity of mobile genes in heterogeneous populations that exhibit fluctuations upon environmental perturbations

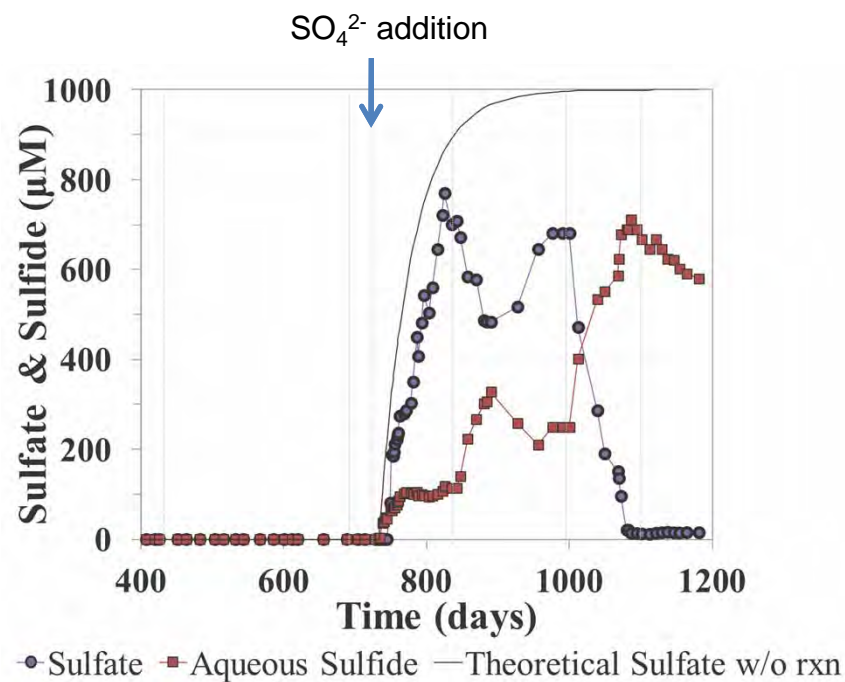
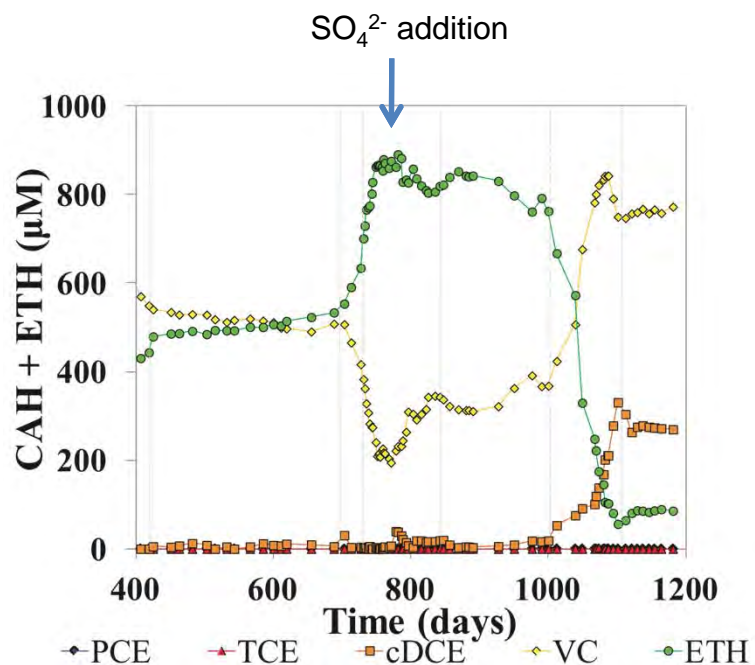


 = *Dehalococcoides* sp.

 = reductive dehalogenase genes

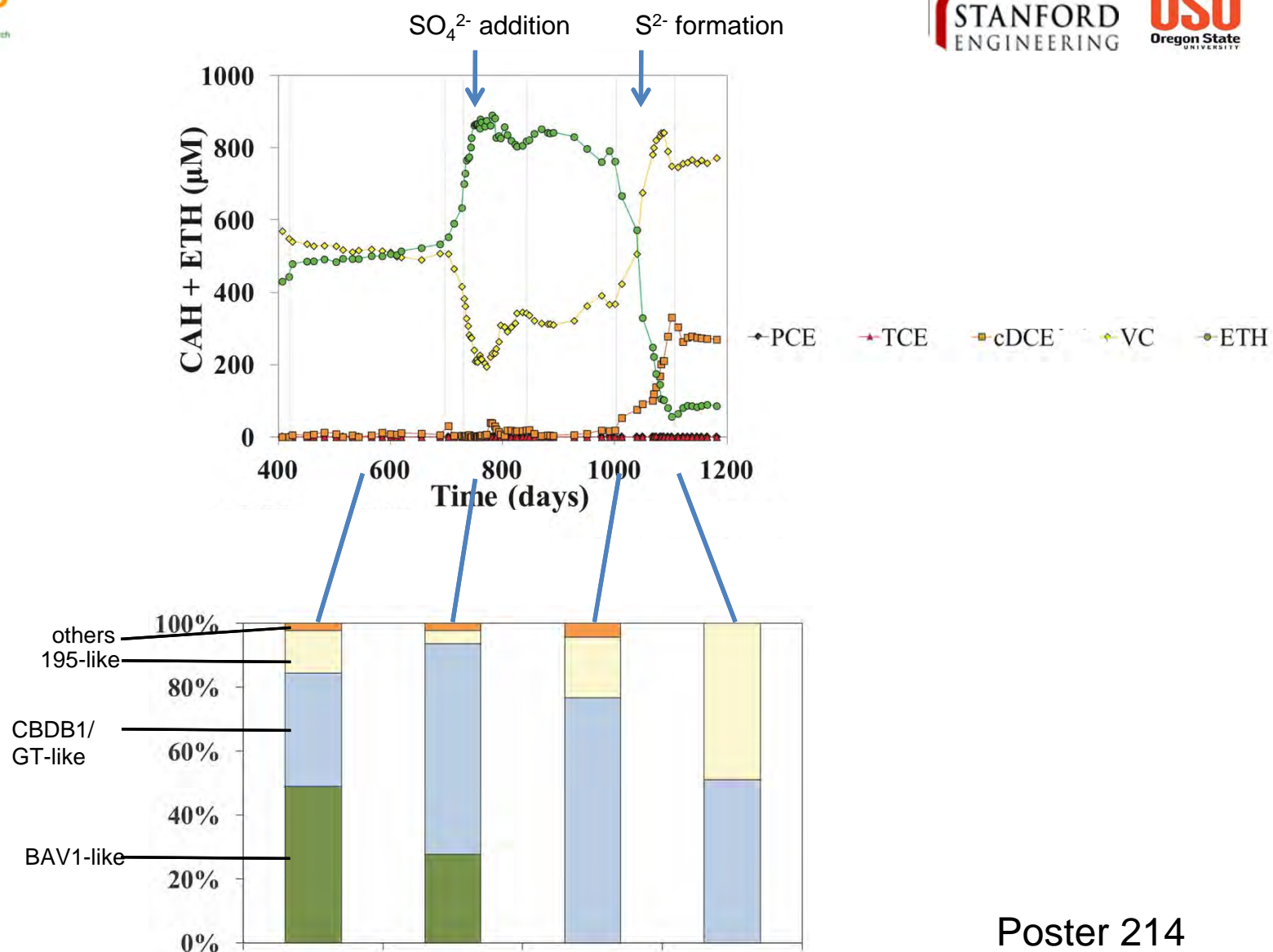
How do competing *electron acceptors* effect Dhc populations?

How do competing *electron acceptors* effect Dhc populations?



Poster 214

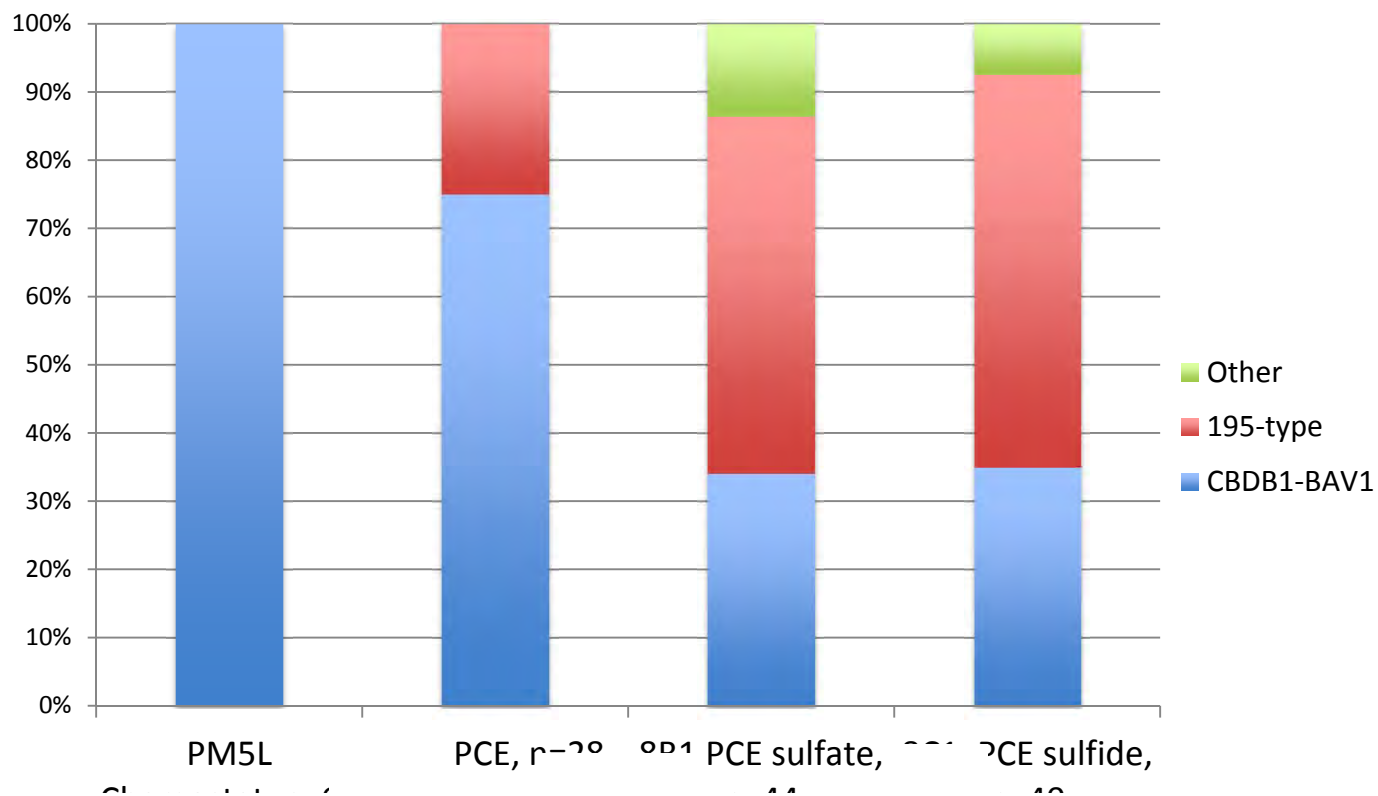
Marshall et al. 2011. *submitted*



Poster 214

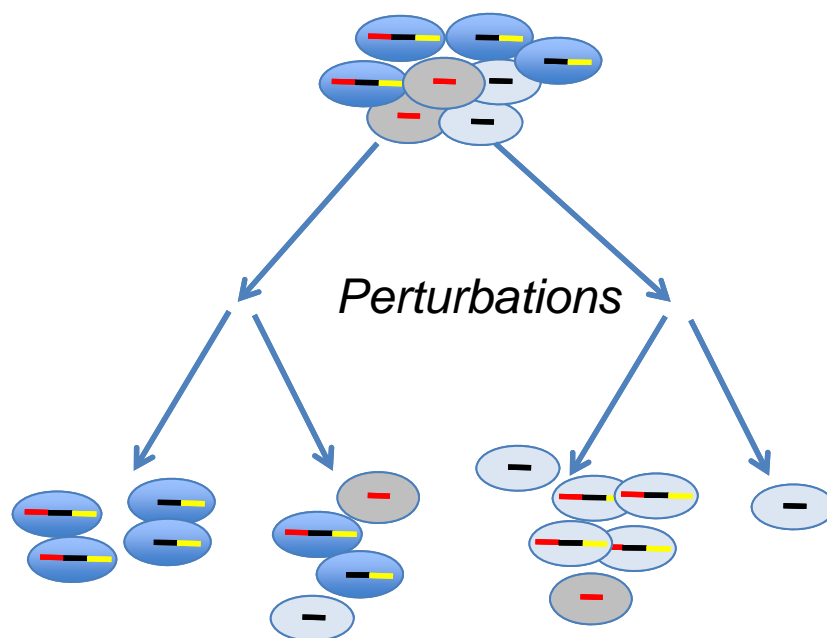
Dynamic change in *Dehalococcoides* population upon alternate acceptor addition

PM5L chemostat batch cultures (sulfide vs. sulfate addition experiments)



Sulfide addition is sufficient to explain *Dehalococcoides* population shift

Useful molecular tools reveal a shift in paradigm for reductive dehalogenation in complex environments



The new paradigm

Chloroethene bioremediation is largely due to dynamic, evolving, and heterogeneous populations of dehalogenating *Chloroflexi* that respond differentially to environmental perturbations.

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